Journal of Animal Science, 2019, 4362–4369

doi:10.1093/jas/skz289
Advance Access publication September 5, 2019
Received: 7 June 2019 and **Accepted:** 2 September 2019
Ruminant Nutrition

RUMINANT NUTRITION

Vitamin E supplementation strategies during feedlot receiving: effects on beef steer performance, antibody response to vaccination, and antioxidant defense[1]

Erin L. Deters and Stephanie L. Hansen[2]

Department of Animal Science, Iowa State University, Ames, IA 50011

[1]This study was partially supported by DSM Nutritional Products, Heerlen, The Netherlands.

[2]Corresponding author: slhansen@iastate.edu

Abstract

This study utilized 204 Angus-based beef steers (249 ± 23 kg SD) from a single ranch with initial serum α-tocopherol concentrations of 3.9 ± 1.0 mg/L to determine the effect of varying doses of vitamin E (**VE**) on feedlot performance, antibody response to vaccination, and antioxidant defense. Seven days after arrival, steers were blocked by body weight and weaning protocol (preweaned, unweaned heavy, and unweaned light) and randomly assigned to pens within blocks (12 pens per block). Preweaned steers had been weaned for approximately 35 d prior to arrival, and unweaned steers were weaned when leaving the origin ranch. Pens within block were randomly assigned to supplemental VE (ROVIMIX E-50 Adsorbate, DSM Nutritional Products, Heerlen, The Netherlands) treatments (n = 9 pens per treatment): no supplemental VE (**CON**), 25 IU/kg dry matter (**DM**; **LOW**), 500 IU per steer daily (**MED**), or 1,000 IU per steer daily (**HIGH**). Back-calculated supplemental VE intake was 0, 151 (24.8 IU/kg DM), 484, and 995 IU/d for CON, LOW, MED, and HIGH, respectively. On day 6, all steers received a booster vaccine against bovine viral diarrhea virus (**BVDV**; Bovi-Shield Gold, One Shot, Zoetis, Parsippany, NJ). Steers were weighed on day –1, 0, 14, 26, and 27. One steer per pen representative of the average body weight of the pen was chosen as a sampling animal for blood (day –1, 6, 14, 26, and 28) and liver (day –3 and 24). Data were analyzed as a randomized complete block design using Proc Mixed of SAS with pen as the experimental unit and the fixed effects of treatment and block. Linear, quadratic, and cubic contrast statements were constructed using Proc IML; morbidity data were analyzed using Proc Glimmix. Day 24 liver and day 26 serum α-tocopherol concentrations were linearly increased by supplemental VE ($P < 0.01$). Supplemental VE did not affect DM intake, average daily gain, or gain:feed from day 0 to 27 ($P \geq 0.37$), or the percentage of steers treated for respiratory disease ($P \geq 0.44$). Day 24 liver glutathione concentrations decreased linearly due to supplemental VE ($P \leq 0.02$). Total- and Mn-superoxide dismutase activities were quadratically affected by supplemental VE ($P \leq 0.07$), with MED steers exhibiting the greatest activity. Over time, BVDV type 1 and 2 antibody titers numerically decreased, whereas the decrease in BVDV type 1 titers was lesser for HIGH steers (linear $P = 0.04$). Increasing doses of VE improved VE status but did not affect overall receiving period performance in steers with minimal to adequate VE status upon arrival.

Key words: antioxidants, immune function, receiving cattle, vitamin E

Introduction

Beef cattle experience numerous stressors prior to and upon feedlot receiving including recent weaning, commingling, transportation, and vaccination. Stressors associated with feedlot receiving have been shown to stimulate an acute inflammatory response (Arthington et al., 2003; Marques et al., 2012) and increase markers of oxidative stress (Chirase et al., 2004), both of which have been negatively associated with animal health and performance (Iqbal et al., 2005; Lykkesfeldt and Svendsen, 2007; Cooke, 2017). Transit stress decreases circulating concentrations of vitamin E (**VE**; Han et al., 1999), a fat-soluble antioxidant that is required for optimal humoral and cell-mediated immunity (Pekmezci, 2011).

The current recommended dose of VE during feedlot receiving is 400 to 500 IU per animal daily (1.6 to 2.0 IU/kg body weight; **BW**), and then 25 to 35 IU/kg dry matter (**DM**; 0.52 to 0.73 IU/kg of BW) for the remainder of the feeding period (NASEM, 2016). However, supplementing VE at ≥400 IU per animal daily tended to increase weight gain and tended to decrease bovine respiratory disease morbidity in feedlot cattle (Secrist et al., 1997). Stressed cattle may benefit from supplementation of VE at greater doses than what is currently recommended due to a possible increased need for antioxidants to combat oxidative stress during the receiving period. Therefore, the objective of this study was to determine the effects of increasing doses of VE in the diets of newly received beef cattle on feedlot performance, antibody response to vaccination, and antioxidant defense. The hypothesis was that increasing doses of supplemental VE would improve VE and antioxidant status and result in subsequent improvements in humoral immune function and performance.

Materials and Methods

Animals and Experimental Design

All experimental procedures were approved by the Iowa State University Animal Care and Use Committee (#9-17-8609-B).

In October 2017, 220 Angus-based beef steers were transported approximately 7.5 h (685 km) from a single ranch in Nebraska to the Iowa State University Beef Nutrition Farm (Ames, IA). Upon arrival (day –7) steers were offered long-stem grass hay top-dressed with a corn silage-based receiving diet (Table 1). No additional hay was fed after the second full day. On day –4, steers were weighed and received visual and electronic identification tags. Steers that did not meet weight criteria and/or displayed signs of illness were excluded from the study; 204 steers (249 ± 23 kg; 197 ± 15 d of age) were utilized. On day 0, steers were blocked by BW and weaning protocol (3 blocks; $n = 12$ pens per block) and randomly assigned to partially covered concrete pens within blocks. Blocks consisted of: preweaned (5 steers per pen, initial BW = 228 kg), unweaned heavy (6 steers per pen, initial BW = 273 kg), and unweaned light (6 steers per pen, initial BW = 243 kg). Steers in the preweaned block were weaned approximately 35 d prior to arrival while steers in the unweaned blocks were not separated from their dams until transportation to Iowa. Pens within block were then randomly assigned to 1 of 4 supplemental VE (ROVIMIX E-50 Adsorbate, DSM Nutritional Products, Heerlen, The Netherlands) treatments ($n = 9$ pens per treatment in total): no supplemental VE (**CON**), VE at 25 IU/kg DM (**LOW**), VE at 500 IU per steer daily (**MED**), or VE at 1,000 IU per steer daily (**HIGH**). Treatments were selected to represent current recommendations for feedlot cattle (LOW; NASEM, 2016), for stressed feedlot cattle (MED; NASEM, 2016), or at a perceived pharmacological dose (HIGH) that may impact aspects of immune function. Individual premixes were made for supplemental VE treatments (LOW, MED, and HIGH) using dried distillers grains as a carrier and premixes were delivered as part of the total mixed ration (**TMR**). Diets were mixed and delivered in the order of CON, LOW, MED, and HIGH and the mixer was flushed with long-stem grass hay between treatments. The LOW premix was delivered at 5% of the diet (DM basis) throughout the trial. Percent inclusions of MED and HIGH treatment premixes were adjusted weekly based on projected treatment group DM intake (**DMI**) to ensure target intake of VE was maintained. Back-calculated supplemental VE intake was 0, 151 ± 28, 484 ± 28, and 995 ± 69 IU/d for CON, LOW, MED, and HIGH, respectively. On a BW basis, back-calculated supplemental VE intake was 0, 0.55, 1.79, and 3.67 IU/kg BW for CON, LOW, MED, and HIGH, respectively.

On day 6, a pour-on doramectin solution (Dectomax, Zoetis, Parsippany, NJ) was administered and steers received a booster vaccine against bovine viral diarrhea virus (**BVDV**) type 1 and 2 (Bovi-Shield Gold, One Shot, Zoetis). Steers were weighed prior to feeding on 2 consecutive days at the beginning (day –1 and 0 = initial BW) and end (day 26 and 27 = final BW) of the trial, as well as on day 6 and 14. Pen DMI, average daily gain (**ADG**), and gain:feed (**G:F**) were calculated from day 0 to 14, 14 to 27, and 0 to 27. Morbidity was assessed daily throughout the course of the study and steers were treated (Draxxin, Zoetis) by farm personnel if visual symptoms (nasal discharge, labored breathing, lethargy, and/or gauntness) were observed and rectal temperature ≥ 39 °C. Due to an outbreak of coccidiosis, Corid (Merial Inc., Duluth, GA) was included in the diet of all steers from day 8 through 12. All steers were subjected to 24-h feed restriction beginning on day 27 and ending on day 28 to determine the effect of supplemental

Table 1. Ingredient composition of the control diet

Dry matter (DM), % as-fed basis	61
Ingredient, % DM basis	
Corn silage	40
Cracked corn	30
DDGS[1]	28.25
Limestone	1.4
Salt	0.31
Rumensin[2]	0.0135
Trace mineral premix[3]	0.025
Analyzed composition[4], %	
Crude protein	15.3
Neutral detergent fiber	25.6
Ether extract	4.8
Calculated composition	
α-Tocopherol[5], IU/kg DM	11.7
Net energy for gain[6], Mcal/kg	1.23

[1]Dried distillers grains with solubles; carrier for micro-ingredients and vitamin E (ROVIMIX E-50 Adsorbate, DSM Nutritional Products, Heerlen, The Netherlands) treatments.
[2]Provided 150 mg monensin per steer daily (Elanco Animal Health, Greenfield, IN).
[3]Provided per kg of diet DM: 10 mg of Cu, 30 mg of Zn, 20 mg of Mn, 0.5 mg of I, 0.1 mg of Se, and 0.1 mg of Co all from inorganic sources and 2,200 IU vitamin A (NASEM, 2016).
[4]Based on total mixed ration analysis from Dairyland, Inc., Arcadia, WI.
[5]Alpha-tocopherol content of corn and corn silage based on values reported by Hidiroglou et al. (1992); α-tocopherol content of DDGS based on values reported by Jung et al. (2013).
[6]Net energy for gain based on NASEM (2016) reported NE_g values of feedstuffs.

VE on the cortisol response elicited by feed restriction (Marques et al., 2012).

Sample Collection and Analytical Procedures

Total feed offered and bunk scores were recorded daily and TMR samples were collected weekly for DM determination by drying in a forced air oven at 70 °C for 48 h. Weekly TMR samples of the CON diet were dried, ground, and composited for analysis of nitrogen (crude protein; AOAC, 1999b; method 990.03), neutral detergent fiber (AOAC, 2005; method 2002.04), and ether extract (AOAC, 1999a; method 920.39) by a commercial laboratory (Dairyland Laboratories, Inc., Arcadia, WI); analyzed compositions are presented in Table 1.

One steer per pen that represented the average weight of the pen was chosen as a sampling animal for liver and blood; the same 36 steers were sampled each time. Blood was collected prior to feeding via jugular venipuncture into vacuum tubes (serum, #366430; sodium heparin, #367874, Becton Dickinson, Franklin Lakes, NJ) on day –1, 6 (prior to vaccination), 14, 26 (prior to feed restriction), and 28 and was transported to the laboratory on ice. Serum tubes remained at room temperature for at least 90 min to allow for coagulation prior to centrifugation at 1,000 × *g* for 10 min at 4 °C; serum was then aliquoted and frozen at –80 °C until further analysis. Serum collected on day –1 and 26 was sent to the Iowa State University Veterinary Diagnostic Laboratory (**ISUVDL**) for analysis of Se concentrations via inductively coupled plasma mass spectrometry (Analytik Jena Inc., Woburn, MA) and α-tocopherol concentrations via high-performance liquid chromatography (**HPLC**). Parameters for HPLC included a mobile phase of 95 (90 methanol/10 chloroform)/5 water, a flow rate of 1 mL/min, a C18 column (Pecosphere, 3 µM, 4.6 × 33 mm; Perkin Elmer, Waltham, MA), and a detection wavelength of 292 nm. Serum collected on day 6, 14, and 26 was sent to the ISUVDL for analysis of BVDV type 1 and 2 antibody titers via virus neutralization (method 9.104; Kalkwarf, 2014). Briefly, serum samples were serially diluted (10^{-1} to 10^{-4}) and incubated with the virus in 96-well culture plates for 2 h at 25 °C. The highest dilution that neutralized 100% of the challenge virus was considered the endpoint. Heparin tubes were centrifuged at 1,000 × *g* for 10 min at 4 °C; plasma was aliquoted and frozen at –80 °C until further analysis. Plasma collected on day –1, 26, and 28 was analyzed for malondialdehyde (**MDA**) concentrations using a commercially available kit (#700870, Cayman Chemical, Ann Arbor, MI); intra- and inter-assay CV were 7.3 and 2.0%, respectively. Plasma collected on day –1, 26, and 28 was also analyzed for cortisol concentrations using a commercially available ELISA kit (#K003-H1/H5, Arbor Assays, Ann Arbor, MI); intra- and inter-assay CV were 4.3 and 10.1%, respectively.

Liver biopsies were performed on day –3 and 24 using a modified method described by Engle and Spears (2000). Briefly, lidocaine was injected, and a small incision made with a scalpel blade between the 11th and 12th ribs on a line from the point of the hip to the point of the shoulder. A modified bone marrow biopsy probe was then inserted into the liver and negative pressure applied with a 10-cc syringe to draw the sample into the probe. Liver samples were snap-frozen in liquid nitrogen and transported to the laboratory where they were stored at –80 °C. Liver samples were ground in liquid nitrogen prior to analysis of α-tocopherol concentrations, superoxide dismutase (**SOD**) activity, and glutathione concentrations. Liver samples were sent to the ISUVDL for α-tocopherol analysis via HPLC using the same parameters as described for serum α-tocopherol. Liver samples for total- and Mn-SOD activity (0.15 g tissue; wet basis) were homogenized in 0.75 mL of 20 mM 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid buffer and centrifuged at 1,500 × *g* for 5 min at 4 °C. The supernatant was then removed, aliquoted, and stored at –80 °C until further analysis (#706002, Cayman Chemical). Intra- and inter-assay CV for Mn-SOD were 5.3 and 8.9%, respectively; intra- and inter-assay CV for total-SOD were 8.8 and 10.1%, respectively. Copper/Zn-SOD activity was calculated by subtracting Mn-SOD from total-SOD activity. Activity is reported as units/mg protein where 1 unit is defined as the amount of enzyme required to dismutate 50% of the superoxide radical. Protein concentrations of the samples analyzed for SOD activity were determined using a commercially available kit (#23200, Thermo Scientific, Rockford, IL); intra- and inter-assay CV were 1.5 and 6.4%, respectively. Liver samples for total (**tGSH**) and oxidized (**GSSG**) glutathione concentrations (0.15 g tissue; wet basis) were homogenized in 0.75 mL of 50 mM 2-(*N*-morpholino)ethanesulfonic acid buffer and prepared for analysis (#703002, Cayman Chemical) as previously described (Hartman et al., 2017). Reduced glutathione (**GSH**) concentrations were calculated by subtracting GSSG from tGSH. Glutathione concentrations are reported as µM/g wet tissue. Intra-assay CV for tGSH and GSSG were 4.9 and 0.5, respectively; inter-assay CV for tGSH and GSSG were 1.8 and 1.1, respectively.

Statistical Analysis

Two LOW steers were removed from the study (1 on day 6, 1 on day 14) due to severe respiratory illness unrelated to treatment; performance data for the subsequent periods were adjusted accordingly. Performance data for 1 MED pen were removed due to overall negative ADG by 1 steer. Data were analyzed using Proc MIXED of SAS 9.4 with pen as the experimental unit ($n = 9$ per treatment). The model included the fixed effect of treatment and block, where block consisted of weaned, unweaned heavy, and unweaned light as described earlier. Orthogonal contrast statements (linear, quadratic, and cubic) were constructed to determine the effects of supplemental VE; contrast coefficients were determined using Proc IML of SAS 9.4 based on back-calculated supplemental VE intakes for individual treatment groups. To account for animal variation at the start of the trial, initial values for serum, plasma, and liver analytes were used as covariates in analysis of subsequent sampling dates. Data were tested for normality and homogeneity of variance using the Shapiro–Wilks test; antibody titers were natural log-transformed to meet the assumption of normality and log-transformed means and SEM are presented. Outliers were determined on a pen basis using Cook's *D* statistic and removed if Cook's $D > 0.05$; 1 pen from LOW was removed from all performance analyses and 1 pen (1 sampler steer) from HIGH was removed from all glutathione and SOD analyses. Morbidity data were analyzed using the GLIMMIX procedure of SAS 9.4 with pen as the experimental unit, the fixed effects of treatment and block, a logit link function, and binomial distribution. Pearson correlations between serum and liver α-tocopherol concentrations were determined using Proc CORR of SAS 9.4. Data are reported as least square means ± SEM. Significance is declared at $P \leq 0.05$ and tendencies from $0.05 < P \leq 0.10$.

Results

Feedlot Performance and Morbidity

Receiving period performance data are presented in Table 2. Initial and final BW were not affected by supplemental VE ($P \geq$

0.75). From day 0 to 14 there was a cubic effect of VE on DMI ($P = 0.01$) driven by greater DMI by LOW and HIGH pens. There was also a tendency for a cubic effect of VE on DMI from day 14 to 27 ($P = 0.10$). A tendency for a quadratic effect of VE on ADG from day 0 to 14 ($P = 0.08$) was observed driven by greater ADG by CON and HIGH pens. From day 14 to 27 there was a quadratic effect of VE on ADG and G:F ($P \leq 0.02$) driven by MED pens exhibiting greater ADG and G:F. There were no effects of VE on DMI, ADG, or G:F from day 0 to 27 ($P \geq 0.37$). All treatments for respiratory disease occurred prior to day 10 of the study and supplemental VE did not affect the percentage of steers treated for respiratory disease ($P \geq 0.44$; Table 2). However, there was a numerical trend for a block effect ($P = 0.11$) where treatment percentages were 0, 2.7, and 13.6% for preweaned, unweaned heavy, and unweaned light, respectively.

Se and VE (α-Tocopherol) Status

Serum Se, serum α-tocopherol, and liver α-tocopherol concentrations are reported in Table 3. Serum Se on day 26 was not affected by supplemental VE ($P \geq 0.56$). Day 26 serum and day 24 liver α-tocopherol concentrations were increased linearly due to supplemental VE ($P < 0.01$). Initial liver (day –3) and serum (day –1) α-tocopherol concentrations were positively correlated ($r = 0.44$; $P = 0.01$) as were final liver (day 24) and serum (day 26) concentrations ($r = 0.69$; $P < 0.01$).

Vaccination and Feed Restriction Response

There was no effect of VE on day 14 (8 d after booster vaccine) BVDV type 1 or 2 antibody titers (Table 4; $P \geq 0.17$). Day 26 (20 d after booster vaccine) BVDV type 1 antibody titers were linearly

Table 2. Effect of supplemental vitamin E (VE) on receiving period performance of beef steers

	Supplemental VE treatment[1]					Contrast P-value		
	CON $n = 9$ pens	LOW $n = 8$ pens	MED $n = 8$ pens	HIGH $n = 9$ pens	SEM[2]	Linear	Quadratic	Cubic
VE intake[3], IU/d	0	151	484	995	–	–	–	–
Initial BW[4], kg	248	250	245	247	8.2	0.88	0.84	0.75
Final BW[5], kg	300	301	301	300	4.8	0.96	0.81	0.99
Dry matter intake, kg/d								
Day 0 to 14	5.0	5.2	4.9	5.1	0.08	0.57	0.28	0.01
Day 14 to 27	7.2	7.0	7.1	7.1	0.09	0.98	0.48	0.10
Day 0 to 27	6.1	6.1	6.0	6.1	0.08	0.79	0.37	0.71
Average daily gain, kg/d								
Day 0 to 14	1.92	1.83	1.81	1.92	0.055	0.70	0.08	0.65
Day 14 to 27	1.95	2.01	2.16	2.02	0.061	0.39	0.02	0.53
Day 0 to 27	1.94	1.92	1.98	1.97	0.042	0.39	0.67	0.46
Gain:feed								
Day 0 to 14	0.389	0.356	0.369	0.377	0.013	0.99	0.24	0.13
Day 14 to 27	0.272	0.288	0.304	0.283	0.008	0.39	0.01	0.94
Day 0 to 27	0.319	0.316	0.330	0.322	0.007	0.54	0.38	0.38
Treated[6], %	4.3	9.4	6.7	6.7	15.6	0.84	0.68	0.44

[1]VE = vitamin E (ROVIMIX E-50 Adsorbate, DSM Nutritional Products, Heerlen, The Netherlands); CON = control (no supplemental VE); LOW = VE at 25 IU/kg DM; MED = VE at 500 IU per steer daily; HIGH = VE at 1,000 IU per steer daily.
[2]Highest SEM of any treatment reported.
[3]Back-calculated supplemental VE intake per steer daily.
[4]Initial body weight (BW) = average of BW collected on day –1 and 0.
[5]Final BW = average of BW collected on day 26 and 27.
[6]Percentage of steers treated for respiratory illness.

Table 3. Effect of supplemental vitamin E (VE) on serum Se and α-tocopherol and liver α-tocopherol concentrations of beef steers

	Supplemental VE treatment[1]					P-value		
	CON	LOW	MED	HIGH	SEM	Linear	Quadratic	Cubic
Serum Se, μg/L								
Day –1[2]	75.2	75.8	76.2	70.4	–	–	–	–
Day 26	96.9	98.0	94.0	97.1	3.65	0.90	0.56	0.58
Serum α-tocopherol, mg/L								
Day –1[2]	4.0	3.5	4.2	3.9	–	–	–	–
Day 26	2.7	3.4	4.6	5.8	0.26	<0.01	0.20	0.81
Liver α-tocopherol, mg/kg wet tissue								
Day –3[2]	11.0	8.5	10.3	11.1	–	–	–	–
Day 24	6.4	8.7	10.1	15.2	0.86	<0.01	0.70	0.26

[1]VE = vitamin E (ROVIMIX E-50 Adsorbate, DSM Nutritional Products, Heerlen, The Netherlands); CON = control (no supplemental VE); LOW = VE at 25 IU/kg DM; MED = VE at 500 IU per steer daily; HIGH = VE at 1,000 IU per steer daily.
[2]Values from day –1 and –3 (prior to treatment initiation) were utilized as a covariate in analysis.

Table 4. Effect of supplemental vitamin E (VE) on bovine viral diarrhea virus (BVDV) type 1 and 2 antibody titers of beef steers

	Supplemental VE treatment[1]					Contrast P-value		
	CON	LOW	MED	HIGH	SEM	Linear	Quadratic	Cubic
BVDV type 1[2]								
Day 6[3] (0)[4]	2.3	3.0	3.6	2.4	–	–	–	–
Day 14 (8)	1.7	1.7	1.4	2.1	0.24	0.32	0.17	0.47
Day 26 (20)	1.5	2.1	2.0	2.5	0.33	0.04	0.77	0.29
BVDV type 2[2]								
Day 6[3] (0)[4]	4.6	5.3	5.2	5.0	–	–	–	–
Day 14 (8)	3.7	3.9	4.1	3.9	0.34	0.61	0.47	0.91
Day 26 (20)	4.2	4.5	4.2	4.6	0.31	0.42	0.72	0.32

[1]VE = vitamin E (ROVIMIX E-50 Adsorbate, DSM Nutritional Products, Heerlen, The Netherlands); CON = control (no supplemental VE); LOW = VE at 25 IU/kg DM; MED = VE at 500 IU per steer daily; HIGH = VE at 1,000 IU per steer daily.
[2]Natural log-transformed; transformed means and SEM presented.
[3]Blood was collected prior to administration of a booster vaccine (Bovi-Shield Gold, One Shot, Zoetis) on day 6 of the study; values from day 6 were utilized as a covariate in analysis.
[4]Day relative to vaccination indicated in parentheses.

Table 5. Effect of supplemental vitamin E (VE) on plasma cortisol and malondialdehyde (MDA) concentrations of beef steers

	Supplemental VE treatment[1]					Contrast P-value		
	CON	LOW	MED	HIGH	SEM	Linear	Quadratic	Cubic
Cortisol, ng/mL								
Day –1[2]	28.9	29.4	25.0	26.2	–	–	–	–
Day 26	20.0	18.2	19.0	21.1	3.32	0.68	0.67	0.78
Day 28[3]	25.5	19.6	24.0	20.6	2.73	0.49	0.98	0.11
MDA, µM								
Day -1[2]	7.7	7.1	6.8	6.6	–	–	–	–
Day 26	7.1	7.6	8.5	7.5	0.61	0.65	0.10	0.86
Day 28[3]	5.0	6.7	7.4	7.2	0.45	0.01	0.01	0.18

[1]VE = vitamin E (ROVIMIX E-50 Adsorbate, DSM Nutritional Products, Heerlen, The Netherlands); CON = control (no supplemental VE); LOW = VE at 25 IU/kg DM; MED = VE at 500 IU per steer daily; HIGH = VE at 1,000 IU per steer daily.
[2]Values from day –1 (prior to treatment initiation) were utilized as a covariate in analysis.
[3]Blood collected after steers were restricted from feed for 24 h.

increased due to supplemental VE ($P = 0.04$); however, there was no effect of VE on day 26 BVDV type 2 antibody titers ($P \geq 0.32$). There was no effect of VE on day 26 (pre-restriction) or day 28 (post-restriction) plasma cortisol concentrations ($P \geq 0.11$; Table 5). A tendency for a quadratic effect of VE on day 26 plasma MDA concentrations ($P = 0.10$) was observed with MED steers having the greatest MDA concentrations. Additionally, there was a quadratic effect of VE on day 28 plasma MDA concentrations ($P = 0.01$) driven by lesser MDA concentrations for CON.

Liver Antioxidants

Liver glutathione and SOD data are presented in Table 6. Day 24 total, oxidized, and reduced glutathione concentrations decreased linearly with increasing supplemental VE ($P \leq 0.02$). There was no effect of VE on the ratio of oxidized to reduced glutathione on day 24 ($P \geq 0.39$). Supplemental VE had a quadratic effect on day 24 total-SOD activity ($P = 0.03$) and tended to have a quadratic effect on day 24 Mn-SOD activity ($P = 0.07$), driven by MED steers exhibiting the greatest activity. Copper/Zn-SOD activity and the ratio of Mn- to total-SOD activity were not affected by supplemental VE on day 24 ($P \geq 0.38$).

Discussion

Beef cattle experience various stressors during the feedlot receiving period that can result in inflammation (Arthington et al., 2003; Marques et al., 2012) and oxidative stress (Chirase et al., 2004) which may hinder cattle health and performance after arrival at the feedlot. Additionally, stress has been shown to negatively impact VE status of cattle (Nockels et al., 1996; Han et al., 1999). Vitamin E is required for oxidative stress protection and optimal immune function, suggesting the feedlot receiving period may be a time when increased dietary supplementation of VE is warranted. The VE supplementation strategies utilized in the present study represent the current recommendation for feedlot cattle (25 to 35 IU/kg DMI; back-calculated intake = 24.8 IU/kg DM; NASEM, 2016), the recently established VE recommendation for stressed cattle (400 to 500 IU per animal daily; back-calculated intake = 484 IU per steer daily; NASEM, 2016), and a pharmacological dose (1,000 IU per animal daily; back-calculated intake = 995 IU per steer daily). These dietary treatments likely represent the majority of VE supplementation strategies utilized in feedlot receiving diets (Samuelson et al., 2016).

Vitamin E is stored in both a fixed pool (adipose tissue) that is mobilized slowly as well as labile pools (plasma and liver) which are depleted rapidly when dietary VE is limiting (Machlin et al., 1979). Serum and liver α-tocopherol concentrations were positively correlated ($r \geq 0.44$) in the present study and were reflective of dietary VE treatments. Serum α-tocopherol concentrations <2.0, 2.0 to 3.0, 3.0 to 4.0, and >4.0 mg/L are considered deficient, marginal, minimal but adequate, and

Table 6. Effect of supplemental vitamin E (VE) on liver glutathione concentrations (μM/g of wet tissue) and superoxide dismutase activity (units/mg protein; 1 unit = the amount of enzyme required to dismutate 50% of the superoxide radical) of beef steers

	Supplemental VE treatment[1]					Contrast *P*-value		
	CON	LOW	MED	HIGH	SEM[2]	Linear	Quadratic	Cubic
Glutathione								
Day –3[3]								
Total	2.04	1.84	2.05	2.02	–	–	–	–
Oxidized	0.37	0.35	0.35	0.34	–	–	–	–
Reduced	1.68	1.49	1.70	1.68	–	–	–	–
Ratio[4]	0.232	0.251	0.223	0.215	–	–	–	–
Day 24								
Total	2.24	2.11	1.84	1.77	0.126	0.01	0.25	0.85
Oxidized	0.40	0.37	0.34	0.32	0.021	<0.01	0.32	0.80
Reduced	1.83	1.74	1.50	1.46	0.116	0.02	0.28	0.79
Ratio[4]	0.223	0.217	0.234	0.214	0.0135	0.76	0.39	0.45
Superoxide dismutase								
Day -3[3]								
Total	162	159	141	148	–	–	–	–
Mn	124	118	105	119	–	–	–	–
Cu/Zn	38.3	40.4	35.2	28.3	–	–	–	–
Ratio[5]	0.78	0.75	0.75	0.81	–	–	–	–
Day 24								
Total	135.2	150.2	162.3	139.7	9.4	0.90	0.03	0.90
Mn	106.5	120.4	122.0	105.7	7.5	0.61	0.07	0.53
Cu/Zn	29.3	29.2	37.0	37.1	6.43	0.63	0.63	0.68
Ratio[5]	0.77	0.80	0.79	0.75	0.037	0.38	0.46	0.70

[1]VE = vitamin E (ROVIMIX E-50 Adsorbate, DSM Nutritional Products, Heerlen, The Netherlands); CON = control (no supplemental VE); LOW = VE at 25 IU/kg DM; MED = VE at 500 IU per steer daily; HIGH = VE at 1,000 IU per steer daily.
[2]Highest SEM of any treatment reported.
[3]Values from day –3 (prior to treatment initiation) utilized as a covariate in analysis.
[4]Ratio = oxidized/reduced glutathione.
[5]Ratio = Mn-SOD/total-SOD.

adequate, respectively (Adams, 1982) and steers began the study with minimal to adequate VE status (3.9 ± 1.0 mg/L) regardless of assigned treatment. By the end of the 27-d trial CON steers had marginal serum α-tocopherol concentrations (2.7 mg/L), LOW steers maintained their minimal status (3.4 mg/L), while MED and HIGH steers had increased serum α-tocopherol concentrations (4.6 and 5.8 mg/L, respectively) above the threshold for adequacy. These data suggest even cattle with adequate VE status may experience a rapid decline in VE status upon arrival due to the stress of feedlot receiving and subsequent increased demand for VE. In agreeance, Carter et al. (2005) observed a decrease in serum α-tocopherol concentrations of receiving cattle from 5.7 mg/L on day 0 to 1.1 mg/L on day 28 when VE was not supplemented in the diet. In the present study, VE supplemented at current recommendations for feedlot cattle (NASEM, 2016) was adequate to prevent a decline in VE status.

Despite changes in VE status, overall receiving period performance did not differ between treatments. Previous studies have observed inconsistent performance responses to VE supplementation during the receiving period. Secrist et al. (1997) reviewed 5 studies regarding the impact of supplemental VE on performance of calves during the first month after transport and found ADG and feed efficiency tended to improve when supplemental VE (450 to 1,400 IU per animal daily) was provided. More recently, Elam (2007) analyzed the results of 7 receiving studies with supplemental VE ranging from 0 to 2,000 IU per animal daily and found no predictive relationship for VE supplementation on DMI, ADG, or G:F. Responses to VE supplementation observed among individual trials were highly variable likely due to many factors including stress experienced by calves, previous diet, duration of VE supplementation, and initial VE status. Steers in the current study were not deficient in VE to start or end the trial, even those that received no supplemental VE, which may have contributed to the lack of performance response. However, it is unclear how prior status affects cattle response to supplemental VE as previous studies seldom report indicators of VE status, possibly due to the assumption that calves with previous exposure to green pastures have adequate VE status upon arrival at the feedlot (Secrist et al., 1997).

The inconsistency of performance responses to VE supplementation indicates the primary objective for greater VE supplementation for receiving cattle may be to support immune system function, primarily via antioxidant protection of immune cells from free radicals released during phagocytosis of pathogens (Babior, 1984). Vitamin E deficiency has been shown to compromise phagocytic, bactericidal, and chemotactic responses of immune cells as well as suppress lymphocyte production, impair T-cell function, and decreased antibody production (Pekmezci, 2011). Alternatively, animals consuming diets that contain more than 5 times the recommended daily allowance of VE have exhibited increased humoral and cell-mediated immune responses (Tengerdy et al., 1973; Tanaka et al., 1979). Rivera et al. (2002) observed a linear increase in ovalbumin antibody titers 21 d after calves received an ovalbumin vaccine, with the greatest antibody response observed in calves receiving supplemental VE at 1,140 IU/d. In the current study, a booster vaccine against BVDV (administered on day 6) was used to elicit an antibody response and determine the effects of varying levels of supplemental VE on antibody concentrations. However,

regardless of treatment, BVDV type 1 and 2 antibody titers numerically decreased over time (day 14 and 26). This lack of antibody response could be a result of increased initial antibody titers from a previous vaccination that occurred at the ranch of origin or the duration of sampling (20-d post-vaccination) was insufficient to capture an increase in antibody titers that may have occurred later. The decrease in BVDV type 1 antibody concentrations over time was numerically less for steers receiving supplemental VE at 1,000 IU/d.

The meta-analysis conducted by Elam (2007) suggests for every 100 IU increase in VE intake per day, a 0.35% decrease in morbidity would be expected. However, the percentage of steers treated for respiratory disease was not affected by supplemental VE in the current study. Since all respiratory treatments occurred prior to day 10 of the study, supplemental VE may not have been fed long enough by that time to influence morbidity rates. Additionally, though the present study had in excess of 200 steers, illness rates were not high. Carter et al. (2005) studied the effect of supplementing VE at 2,000 IU per animal daily for 0, 7, 14, or 28 d and observed a numeric decrease in morbidity for calves supplemented for 14 or 28 d, suggesting the positive effects of VE on health might be time-dependent. Although VE did not affect morbidity in the present study, there was a trend for a block effect where treatment percentages were least for preweaned calves and greatest for lightweight unweaned calves. These data confirm that weaning cattle prior to feedlot entry and heavier BW are critical factors in decreasing morbidity upon arrival. Age is also an important factor as yearlings may be expected to have lower morbidity rates during the receiving period than cattle that enter the feedlot as calves.

The current study also sought to determine the effects of supplementing an exogenous antioxidant (i.e., VE) on endogenous components of the antioxidant defense system including glutathione and SOD. It is important to note that Se and VE both have antioxidant functions and can therefore spare each other. However, serum Se concentrations were not affected by supplemental VE and steers had adequate Se status to start and end the trial (Herdt and Hoff, 2011) indicating Se status had minimal influence on the antioxidant measures discussed herein. Glutathione concentrations were linearly decreased due to supplemental VE, possibly due to a sparing effect where supplementation of an exogenous antioxidant resulted in less signaled need for endogenous antioxidant production. The synthesis of glutathione has also been shown to be upregulated in response to VE deficiency (Morante et al., 2005). Thus, it is possible that supplementing VE at high concentrations could have had the opposite effect and directly inhibited glutathione synthesis. Steers supplemented VE at 500 IU per daily had the greatest activity of the antioxidant enzyme SOD, driven primarily by changes in the Mn-dependent isoform. A previous study in rats revealed an increase in Mn-SOD mRNA expression and activity after 4 wk of twice weekly intraperitoneal injections of VE at greater doses (30 or 100 mg/kg BW) vs. lesser doses (0 or 10 mg/kg BW; Hajiani et al., 2013). However, the greatest VE dose in the current study (1,000 IU per steer daily) resulted in Mn-SOD activity similar to CON steers. This could be due to the prooxidant capabilities of VE when there is a lack of other antioxidants, such as vitamin C and glutathione, to reduce the α-tocopherol radical (Rietjens et al., 2002). The decreased glutathione concentrations discussed previously suggest that the pharmacological VE dose may have contributed to prooxidant conditions and subsequent inactivation of Mn-SOD (Yamakura and Kawasaki, 2010).

Steers in the current study were subjected to 24 h of feed restriction to elicit a cortisol response and determine if supplemental VE affected this response. However, cortisol concentrations were not increased post-restriction. There was also no effect of supplemental VE on pre- or post-restriction cortisol concentrations while Reddy et al. (1987) observed lesser serum cortisol concentrations in dairy calves supplemented with 125, 250, or 500 IU of VE per calf daily compared to nonsupplemented calves. In addition to stimulating a cortisol response, it was hypothesized that feed restriction would increase concentrations of MDA, a marker of lipid peroxidation, and VE would mitigate this increase due to its role as a lipid soluble antioxidant. However, MDA concentrations were numerically lesser for all treatment groups post-restriction and MED steers exhibited the greatest plasma MDA concentrations. Cusack et al. (2005) also observed greater plasma MDA concentrations in heifer calves supplemented VE at 822 IU/d and suggested that this response may be partially due to MDA being a normal intermediate formed during biosynthesis of eicosanoids, lipid signaling molecules involved in immunity and inflammation (Harizi et al., 2008). It has also been suggested that the method used to analyze MDA concentrations in the current study (thiobarbituric acid reactive substances) may contribute to sample oxidation during analysis and may not be specific enough due to the ability of thiobarbituric acid to react with a variety of compounds (Del Rio et al., 2005).

Despite linear increases in circulating VE and VE stores, supplemental VE had no effect on steer morbidity and minimal effects on growth performance in the current study. However, other variables of interest were differentially affected by VE supplementation. For example, supplementation of VE at the recommended rate for feedlot cattle (25 IU/kg DMI; NASEM, 2016) prevented the decline in VE status observed in control steers while supplementation of VE at the recommended rate for stressed cattle (500 IU per steer daily; NASEM, 2016) increased activity of the antioxidant enzyme SOD. Additionally, supplementation of VE at a pharmacological dose (1,000 IU per steer daily) lessened the decrease in BVDV antibody titers over time but decreased concentrations of the endogenous antioxidant glutathione, indicating VE supplementation rate may differentially affect variables of interest to feedlot producers. The responses to VE supplementation observed herein were likely impacted by initial VE status (minimal to adequate) and lack of VE deficiency, even in CON steers. Therefore, the physiological needs of the animal (e.g., growth, immune function, antioxidant defense) and VE status upon arrival at the feedlot should be taken into consideration when defining recommended VE supplementation rates for receiving cattle.

Literature Cited

Adams, C. R. 1982. Feedlot cattle need supplemental vitamin E. *Feedstuffs*. **54**:24–25.

AOAC. 1999a. Ether extract in animal feeds. Method 920.39. In: Cunniff P., editor. *Official methods of analysis*. 16th ed. Vol. 1. Assoc. Off. Anal. Chem., Gaithersburg, MD. Ch. 4. p. 25.

AOAC. 1999b. Protein (crude) in animal feed: combustion method. Method 990.03. In: Cunniff P., editor. *Official methods of analysis*. 16th ed. Vol. 1. Assoc. Off. Anal. Chem., Gaithersburg, MD. Ch. 4. p. 18–19.

AOAC. 2005. Amylase-treated neutral detergent fiber in feeds. Method 2002.04. In: Horwitz W., editor. *Official methods of analysis*. 18th ed. Assoc. Off. Anal. Chem., Gaithersburg, MD. Ch. 4. p. 49–55.

Arthington, J. D., S. D. Eichert, W. E. Kunkle, and F. G. Martin. 2003. Effect of transportation and commingling on the acute-phase protein response, growth, and feed intake

of newly weaned beef calves. *J. Anim. Sci.* **81**:1120–1125. doi:10.2527/2003.8151120x

Babior, B. M. 1984. The respiratory burst of phagocytes. *J. Clin. Invest.* **73**:599–601. doi:10.1172/JCI111249

Carter, J. N., D. R. Gill, C. R. Krehbiel, A. W. Confer, R. A. Smith, D. L. Lalman, P. L. Claypool, and L. R. McDowell. 2005. Vitamin E supplementation of newly arrived feedlot calves. *J. Anim. Sci.* **83**:1924–1932. doi:10.2527/2005.8381924x

Chirase, N. K., L. W. Greene, C. W. Purdy, R. W. Loan, B. W. Auvermann, D. B. Parker, E. F. Walborg, D. E. Stevenson, Y. Xu, and J. E. Klaunig. 2004. Effect of transport stress on respiratory disease, serum antioxidant status, and serum concentrations of lipid peroxidation biomarkers in beef cattle. *Am. J. Vet. Res.* **65**:860–864. doi:10.2460/ajvr.2004.65.860

Cooke, R. F. 2017. Invited paper: nutritional and management considerations for beef cattle experiencing stress-induced inflammation. *Prof. Anim. Sci.* **33**:1–11. doi:10.15232/pas.2016-01573

Cusack, P. M. V., N. P. Mcmeniman, and I. J. Lean. 2005. The physiological and production effects of increased dietary intake of vitamins E and C in feedlot cattle challenged with bovine herpesvirus. *J. Anim. Sci.* **83**:2423–2433. doi:10.2527/2005.83102423x

Del Rio, D., A. J. Stewart, and N. Pellegrini. 2005. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutr. Metab. Cardiovasc. Dis.* **15**:316–328. doi:10.1016/J.NUMECD.2005.05.003

Elam, N. A. 2007. Impact of vitamin E supplementation on newly received calves: a review and meta-analysis. *Prof. Anim. Sci.* **23**:455–458. doi:10.1532/S1080-7446(15)31005-6

Engle, T. E., and J. W. Spears. 2000. Effects of dietary copper concentration and source on performance and copper status of growing and finishing steers. *J. Anim. Sci.* **75**:3074–3081. doi:10.2527/2000.7892446x

Hajiani, M., F. Razi, A. Golestani, M. Frouzandeh, A. Owji, S. Khaghani, N. Ghannadian, A. Shariftabrizi, and P. Pasalar. 2013. Time- and dose-dependent differential regulation of copper-zinc superoxide dismutase and manganese superoxide dismutase enzymatic activity and mRNA level by vitamin E in rat blood cells. *Redox. Rep.* **17**:101–107. doi:10.1179/1351000212Y.0000000008

Han, H., T. C. Stovall, J. T. Wagner, and D. R. Gill. 1999. Impact of Agrado on tocopherol metabolism by transport-stressed heifers. In: *Animal Science Research Report MP-973*. Stillwater, OK: University of Oklahoma. p. 119–125.

Harizi, H., J.-B. B. Corcuff, and N. Gualde. 2008. Arachidonic-acid-derived eicosanoids: roles in biology and immunopathology. *Trends Mol. Med.* **14**:461–469. doi:10.1016/j.molmed.2008.08.005

Hartman, S. J., O. N. Genther-Schroeder, and S. L. Hansen. 2017. Effect of trace mineral source on mineral status and performance of beef steers fed low- or high-sulfur diets. *J. Anim. Sci.* **95**:4139–4149. doi:10.2527/jas.2017.1722

Herdt, T. H., and B. Hoff. 2011. The use of blood analysis to evaluate trace mineral status in ruminant livestock. *Vet. Clin. North Am. Food Anim. Pract.* **27**:255–283. doi:10.1016/j.cvfa.2011.02.004

Hidiroglou, N., N. Cave, A. S. Atwal, E. R. Farnworth, and L. R. Mcdowell. 1992. Comparative vitamin E requirements and metabolism in livestock. *Ann. Rech. Vet.* **23**:337–359. HAL Id: hal-00902095

Iqbal, M., N. R. Pumford, Z. X. Tang, K. Lassiter, C. Ojano-Dirain, T. Wing, M. Cooper, and W. Bottje. 2005. Compromised liver mitochondrial function and complex activity in low feed efficient broilers are associated with higher oxidative stress and differential protein expression. *Poult. Sci.* **84**:933–941. doi:10.1093/ps/84.6.933

Jung, B., A. B. Batal, N. E. Ward, and N. Dale. 2013. Vitamin composition of new-generation corn distillers dried grains with solubles. *J. Appl. Poult. Res.* **22**:71–74. doi:10.3382/japr.2012-00595

Kalkwarf, E. 2014. *Procedure for virus neutralization (VN) test*. Iowa State University Diagnostic Laboratory (ISUVDL), Ames, IA.

Lykkesfeldt, J., and O. Svendsen. 2007. Oxidants and antioxidants in disease: oxidative stress in farm animals. *Vet. J.* **173**:502–511. doi:10.1016/j.tvjl.2006.06.005

Machlin, L. J., J. Keating, J. Nelson, M. Brin, R. Filipski, and O. N. Miller. 1979. Availability of adipose tissue tocopherol in the guinea pig. *J. Nutr.* **109**:105–109. doi:10.1093/jn/109.1.105

Marques, R. S., R. F. Cooke, C. L. Francisco, and D. W. Bohnert. 2012. Effects of twenty-four hour transport or twenty-four hour feed and water deprivation on physiologic and performance responses of feeder cattle. *J. Anim. Sci.* **90**:5040–5046. doi:10.2527/jas.2012-5425

Morante, M., J. Sandoval, J. L. Rodriguez, F. V Paixardo, J. R. Vina, L. Torres, and T. Barber. 2005. Vitamin E deficiency induces liver nuclear factor-KB DNA-binding activity and changes in related genes. *Free. Rad. Res.* **39**:1127–1138. doi:10.1080/10715760500193820

National Academies of Sciences, Engineering, and Medicine (NASEM). 2016. *Nutrient requirements of beef cattle*. 8th rev. ed. Natl. Acad. Press, Washington, DC.

Nockels, C. F., K. G. Odde, and A. M. Craig. 1996. Vitamin E supplementation and stress affect tissue alpha-tocopherol content of beef heifers. *J. Anim. Sci.* **74**:672–677. doi:10.2527/1996.743672x

Pekmezci, D. 2011. Vitamin E and immunity. In: G. Litwack, editor, *Vitamins and hormones*, vol. **86**. Elsevier Inc., London, UK. p. 179–201.

Reddy, P., J. Morrill, H. Minocha, and J. Stevenson. 1987. Vitamin E is immunostimulatory in calves. *J. Dairy Sci.* **70**:993–999. doi:10.3168/jds.S0022-0302(87)80104-2

Rietjens, I. M. C. M., M. G. Boersma, L. De Haan, B. Spenkelink, H. M. Awad, N. H. P. Cnubben, J. J. Van Zanden, H. Van Der Woude, G. M. Alink, and J. H. Koeman. 2002. The pro-oxidant chemistry of the natural antioxidants vitamin C, vitamin E, carotenoids and flavonoids. *Environ. Toxicol. Pharmacol.* **11**:321–333. doi:10.1016/S1382-6689(02)00003-0

Rivera, J. D., G. C. Duff, M. L. Galyean, D. A. Walker, and G. A. Nunnery. 2002. Effects of supplemental vitamin E on performance, health, and humoral immune response of beef cattle. *J. Anim. Sci.* **80**:933–941. doi:10.2527/2002.804933x

Samuelson, K. L., M. E. Hubbert, M. L. Galyean, and C. A. Löest. 2016. Nutritional recommendations of feedlot consulting nutritionists: the 2015 New Mexico state and Texas tech university survey. *J. Anim. Sci.* **94**:2648–2663. doi:10.2527/jas2016-0282

Secrist, D. S., F. N. Owens, and D. R. Gill. 1997. Effects of vitamin E on performance of feedlot cattle: a review. *Prof. Anim. Sci.* **13**:47–54. doi:10.15232/S1080-7446(15)31844-1

Tanaka, J., H. Fujiwara, and M. Torisu. 1979. Vitamin E and immune response. I. Enhancement of helper T cell activity by dietary supplementation of vitamin E in mice. *Immunology* **38**:727–734.

Tengerdy, R. P., R. H. Heinzerling, G. L. Brown, and M. M. Mathias. 1973. Enhancement of the humoral immune response by vitamin E. *Inter. Arch. Allergy Immunol.* **44**:221–232. doi:10.1159/000230931

Yamakura, F., and H. Kawasaki. 2010. Post-translational modifications of superoxide dismutase. *Biochim. Biophys. Acta* **1804**:318–325. doi:10.1016/j.bbapap.2009.10.010